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SYNTHESIS AND BIOLOGICAL EVALUATION OF HYDRAZONE DERIVATIVES

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ABSTRACT

A number of hydrazone derivatives were synthesized, and were purified by crystallization or by column chromatography. Structures of all the synthesized compounds are supported by correct IR, ^{1}H NMR, mass spectral and analytical data. Anti-inflammatory activity evaluation was carried out by using carrageenin-induced paw oedema assay and compounds I, II and III exhibited good anti-inflammatory activity, that is 38%, 37% and 52% at 75 mg/kg po, respectively. Therefore many researchers have synthesized these compounds as target structures and evaluated their biological activities. These observations have been guiding for the development of new hydrazones that possess varied biological activities.

KEY WORDS

Hydrazone, Anti-Inflammatory, Carrageenin, Indomethacin and Column chromatography.

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INTRODUCTION

Hydrazones have been demonstrated to possess, among other, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumoral activities (Figure No.1). For example, isonicotinoyl hydrazones are antitubercular; 4-hydroxybenzoic acid[(5-nitro-2-furyl)methylene]- hydrazide (nifuroxazide) is an intestinal antiseptic; 4-fluorobenzoic acid[(5-nitro-2-furyl)methylene]- hydrazide[1] and 2,3,4- hydrazone[2], which were synthesized in our Department, have

antibacterial activity against both *Staphylococcus aureus* ATCC 29213 and *Mycobacterium tuberculosis* H37Rv at a concentration of 3.13 μg/mL. *N*1-(4 Methoxy benzamido) benzoyl]-*N*2-[(5-nitro-2-furyl)methylene]hydrazine, which was also synthesized in our Department[3], demonstrated antibacterial activity. In addition, some of the new hydrazide-hydrazones that we have recently synthesized were active against the same strain of *M. tuberculosis* H37Rv between the concentrations of 0.78-6.25 μg/mL[4].

Hydrazones containing an azometine -NHN=CH- proton are synthesized by heating the appropriate substituted hydrazines/hydrazides with aldehydes and ketones in solvents like ethanol, methanol, tetrahydrofuran, butanol, glacial acetic acid, ethanol-glacial acetic acid. Another synthetic route for the synthesis of hydrazones is the coupling of aryldiazonium salts with active hydrogen compounds. In addition, 4-acetylphenazone isonicotinoylhydrazones was prepared by Amal and Ergen[5] by exposing an alcohol solution of 4-acetylphenazone and INH to sunlight or by mixing them with a mortar in the absence of the solvent.

Many effective compounds, such as iproniazide (Figure No.1) and isocarboxazide (Figure No.1), are synthesized by reduction of hydrazide-hydrazones. Iproniazide, like INH, is used in the treatment of tuberculosis. It has also displays an antidepressant effect and patients appear to have a better mood during the treatment. Another clinically effective hydrazide-hydrazones is nifuroxazide, which is used as an intestinal antiseptic.

Non Steroidal Anti- inflammatory

Traditional non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain, but their long term use is limited by serious gastrointestinal side effects. Whereas NSAIDs inhibit the two recognised forms of prostaglandin G/H synthase (also referred to as cyclo-oxygenase), selective cyclo-oxygenase-2 (COX 2) inhibitors are selective inhibitors of the COX 2 isozyme[6]. As the anti-inflammatory effects of NSAIDs were believed to be mediated by inhibition of COX 2, and their gastrointestinal side effects by inhibition of COX 1, people hypothesised that selective COX 2 inhibitors would provide a safer alternative to traditional NSAIDs. However, although some studies have reported a lower incidence of upper gastrointestinal complications with selective COX 2 inhibitors than with traditional NSAIDs, recent concerns about the cardiovascular safety of selective COX 2 inhibitors have limited their use.

Although the Vioxx gastrointestinal outcomes research (VIGOR) trial reported a fivefold increase in myocardial infarction among participants allocated to rofecoxib (20 rofecoxib *v* 4 naproxen; $P < 0.001$)[7], this difference might have occurred, at least in part, because high dose naproxen inhibits platelet aggregation throughout the dosing interval. However, the results of the adenomatous polyp prevention on Vioxx (APPROVe) trial, which was the first relatively large trial comparing a selective COX 2 inhibitor with placebo, indicated that rofecoxib increased the risk of vascular events by about twofold[8]. Soon afterwards, the adenoma prevention with celecoxib (APC) trial, comparing celecoxib with placebo, reported a similar excess[9].

The accumulating evidence suggests that selective COX 2 inhibitors are associated with an increased risk of vascular events, but several important questions remain unanswered. Firstly, what is the magnitude of any excess risk of myocardial infarction, stroke, and vascular mortality. Secondly, is the excess risk of vascular events dose related, and is the size of this risk different in people who are also taking aspirin (which chiefly inhibits COX 1 at low doses[10]. Thirdly, are traditional NSAIDs (which also inhibit COX 2) associated with an increased risk of vascular events.

General Synthesis of hydrazide and hydrazone derivatives

Hydrazide-hydrazones compounds are not only intermediates but they are also very effective organic compounds in their own right. When they are used as intermediates, coupling products can be synthesized by using the active hydrogen component of –CONHN=CH- azometine group[11]. *N*-Alkyl hydrazides can be synthesized by reduction of hydrazones with NaBH4, substituted 1, 3, 4-

oxadiazolines can be synthesized when hydrazones are heated in the presence of acetic anhydride[12]. 2-Azetidinones can be synthesized when hydrazones react with trietylamine chloro acetylchloride[13]. 4-Thiazolidinones are synthesized when hydrazones react with thioglycolic acid/ thiolactic acid [14] (Figure No.2).

EXPERIMENTAL PART

IR spectrum was recorded using FT-IR, Perkin Elmer 8400 series instrument. NMR spectrum was obtained on a JEOL 500 MHz Bruker Daltonics, Germany. Mass spectrum was recorded by using Shimadzu MS-2010 A, Koyoto, Japan. Melting points (uncorrected) were obtained on a melting point apparatus, Lab India, Mumbai. Carrageenan was obtained from Sigma Aldrich Co, St Louis, USA. All other chemicals used were of analytical grade.

Compound –I

Synthesis of isatin hydrazones

An appropriate isatin (indole-2, 3-dione) (V, 0.01 mol) was dissolved in alcohol (20 ml) and added hydrazine hydrate (99%, 0.015 mol) while shaking. The reaction mixture was stirred well, warmed on a water-bath for 10 min and left in the refrigerator for 3 h. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold alcohol. The product was dried and purified by recrystallization from chloroform.

M.W: 161, M.F: $C_8H_7N_3O$, M.P 220^0 C. Yield 74.5%.

Compound –II

Synthesis of 2-phenyl 5-benzylidene 3N-(4-acetyl phenyl)-1, 5-dihydro-imidazole-4-one

An equimolar mixture of 4-benzylidine-2-phenyloxazol-5-one and Methyl p-aminobenzoate was heated on oil bath at 140^{0}C for 40-50 min. The resulting jelly like mass was recrystallized from methanol.

M.W: 382, M.F: $C_{24}H_{18}N_2O_3$, M.P: 181^{0}C. IR(KBr in cm^{-1}): 1280 (O-C=O bending), 1712, (C=O streching), 2923 (Ar-CH streching): ^{1}H NMR ($CDCl_3$) (δppm): 3.901 (s, 3H, CH_3), 2.597 (s, 1H, CH), 7-8 (m, 14H, Ar CH); MS: (m/z) 382 (M^+); Analysis ($C_{24}H_{18}N_2O_3$) Cal.(Found)%: C 75.39 (75.35), H 4.7 (4.2), N 7.3 (7.01).

Compound –III

Synthesis of 3[(5-Benzylidene-2-phenyl) -3, 5-dihydro-4H-imidazole-4-one-1-(4-benzoyl hydrazono)]-indole-2-ones

A mixture of equimolar quantity of isatin hydrazones (0.01mol) and 2-phenyl 5-bezylidene 3N (4-acetyl phenyl)-1, 5-dihydro-imidazole-4-one (0.01mol), was dissolved in methanol containing a catalytic amount of potassium hydroxide and heated under refluxed for 5-6 h. The reaction mixture was cooled and neutralized with concentrated hydrochloric acid. The resultant product was then filtered, dried and purified by recrystallization from methanol.

M.W: 546, M.F: $C_{31}H_{20}ClN_5O_3$, M.P: 252^{0}C, IR (KBr in cm^{-1}): 1280.65 1712 (CO streching), 2923 (ArCH streching):, (O-C=O bending), ^{1}H NMR ($CDCl_3$) (δppm): 3.90 (s, 3H, CH_3), 2.1(s, 1H ,CH), 7-8 (m,14H , Ar CH) , MS (m/z): 546 (M^+).

Anti-inflammatory activity by carrageenan induced paw edema in rats

All the animals were acclimatized to laboratory conditions for a week before commencement of the experiment. The experiments were performed during the light portion between 07:00-18:00 h to avoid circadian influences. Animal studies were performed according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India.

Wistor albino rats (150-200 g) were divided into five groups with six animals in each group.

Group I animals were served as control and received 0.5 % w/v dispersion of CMC in distilled water.

Groups II-IV animals were treated with compounds **I**, **II** and **III** at 75 and 150 mg/kg, respectively.

Group V animals were treated with standard indomethacin at 10 mg/kg. All the treatments were administered orally. The initial hind paw volume of rats was determined volumetrically by using a plethysmometer. A solution of carrageenan in CMC (1%, 0.05 ml/rat) was injected subcutaneously into the left hind paw 30 min after the treatments. The animals in the control group received the vehicle

only. Paw volumes were measured up to 6 h at intervals of 30, 60, 120, 180, 240, 300 and 360 min and percent increase in edema between the control and treated groups were compared. The percentage protection was calculated as

$$\%\text{ Paw edema inhibition} = 1 - \frac{\text{Edema volume in drug treated group}}{\text{Edema volume in control group}} \times 100$$

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by multiple comparison using the Dunnet's test. P values < 0.05 were considered as significant.

RESULT AND DISCUSSION

A synthesis (Figure No.2) that results in the clubbing of hydrazone with a suitably modified organic moiety will be important in terms of the biological activity the resultant molecule is expected to display. Against carrageenan induced paw edema in rats, compounds **I**, **II** and **III** at 150 mg/kg significantly reduced the paw edema after 30-360 min when compared to control (Table No.1). Compound **III** exhibited significant activity at 75 mg/kg after the 30-360 min. However, compounds **I, II** and **III** produced significant activity in a dose dependant manner. The standard indomethacin at 10 mg/kg produced better results than the tested samples. Carrageenan induced inflammation is a non-specific inflammation resulting from a complex of diverse mediators. This model is conventional, sensitive, accepted for screening of newer anti-inflammatory agents and reliably predicts the anti-inflammatory efficacy based on inhibition of prostaglandin amplification. In the present study, compounds **I, II** and **III** exhibited potent effect indicating it to be a good candidate for anti-inflammatory activity. Further research would be of interest to explain the exact mechanism of these compounds and toxicity studies can also be explored.

Table No.1: Anti-inflammatory activity of hydrazone derivatives

S.No	Treatment	Dose (mg/kg)	Mean increase in rat paw edema (ml)			
			0.5h	1h	2h	3h
1	Control vehicle	3 ml	0.57±0.02	0.62±0.03	0.69±0.02	0.78±0.02
2	COM – I	75	0.50±0.03	0.52±0.04	0.64±0.02	0.68±0.02
		150	0.49±0.04	0.54±0.05	0.60±0.03	0.64±0.01
3	COM- II	75	0.47±0.01	0.50±0.01	0.54±0.01	0.57±0.02
		150	0.40±0.02	0.44±0.02	0.48±0.02	0.52±0.01
4	COM – III	75	0.39±0.03	0.41±0.02	0.40±0.01	0.40±0.08
		150	0.36±0.02	0.38±0.04	0.37±0.02	0.34±0.06
5	Indomethacin	10	0.28±0.03	0.26±0.05	0.30±0.03	0.22±0.04

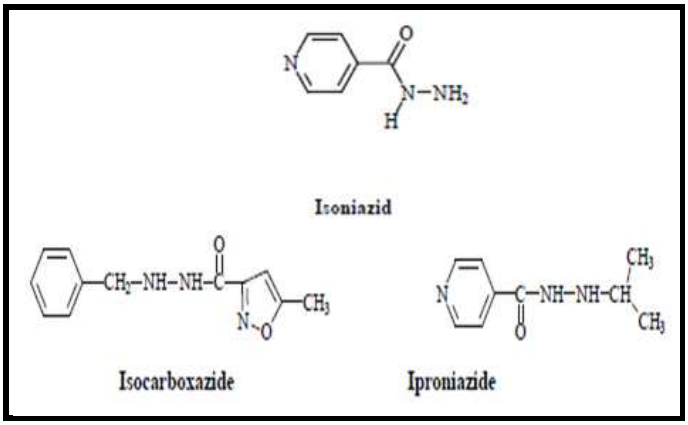

Figure No.1: Structure of Isoniazid

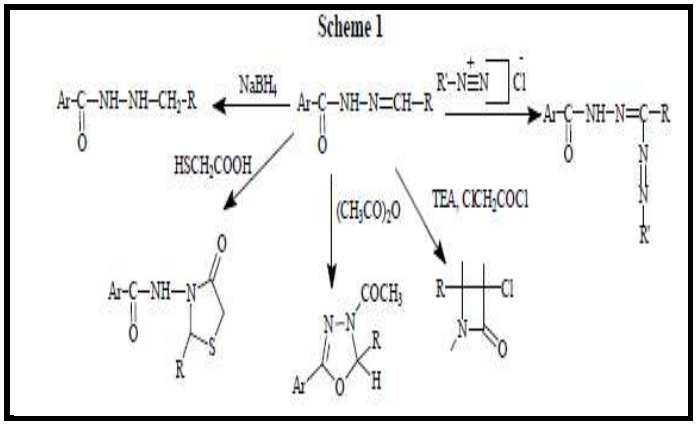

Figure No.2: Synthesis of hydrazone derivatives

CONCLUSION

The yield of all hydrazone derivatives were found to be in the range of 38-74%. The purity of compounds was ascertained by melting point and TLC. The assigned structure was further established by IR, 1HNMR and elemental analysis studies. The acute anti-inflammatory activity of the synthesized compounds was screened using the carrageenan induced paw oedema method in rats. Indomethacin was used as a reference drug. In the prepared hydrazone all compounds showed significant action, when compared to the standard drug Indomethacin. But the compound III, exhibited highest anti-inflammatory activity. From the present study, it may be concluded that the hydrazone compounds can potentially be developed into useful anti- inflammatory agents that can prompt future researchers to synthesize a series of hydrazone derivatives containing a wide variety of substituents with the aim of obtaining novel heterocyclic systems with enhanced activity. Further work to develop and improve similar and related compounds and test them for manifold biological activity
is in progress.

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